FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/554, 33/569 — A1

(11) International Publication Number: WO 97/03360

(43) International Publication Date: 30 January 1997 (30.01.97)

(21) International Application Number: PCT/US96/11245

(22) International Filing Date: 2 July 1996 (02.07.96)

(30) Priority Data:
08/499,422 7 July 1995 (07.07.95) US

(71) Applicant: ORAVAX, INC. [US/US]; 38 Sidney Street, Cambridge, MA 02139-4169 (US).

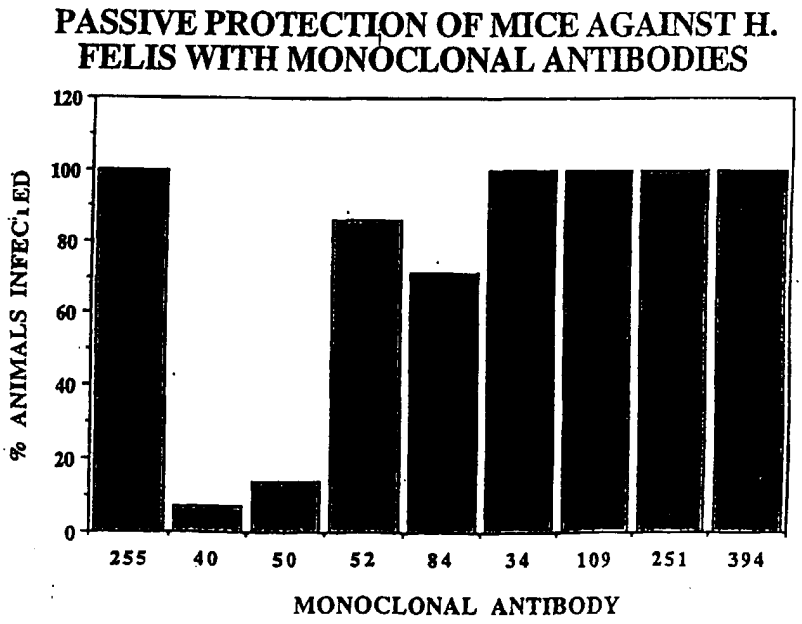
(72) Inventors: NEDRUD, John, G.; 3196 Warrington Road, Shaker Heights, OH 44120 (US). CZINN, Steven, J.; 2388 Kenilworth Road, Cleveland Heights, OH 44106 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: HELICOBACTER VACCINE ANTIGEN RECOGNIZED BY A PROTECTIVE MONOCLONAL ANTIBODY (IgG 50)



(57) Abstract

The invention features *Helicobacter* polypeptides which are recognized by monoclonal antibody IgG 50, monoclonal antibodies (e.g., IgG 50) which recognize these polypeptides and methods of preventing and/or treating *Helicobacter* infection using these polypeptides and monoclonal antibodies. The figure is a graph showing that monoclonal antibody IgG 50 is effective at passive protection of mice against *Helicobacter felis* infection.

HELICOBACTER VACCINE ANTIGEN RECOGNIZED BY A PROTECTIVE MONOCLONAL ANTIBODY (IgG 50)

Background of the Invention

This invention relates to methods and compositions for preventing and/or treating *Helicobacter* infection.

Helicobacter is a genus of spiral, gram-negative bacteria which colonize the gastrointestinal tracts of mammals. Several species colonize the stomach, most notably, *H. pylori*, *H. heilmanii*, *H. felis*, and *H. mustelae*. Although *H. pylori* is the species most commonly associated with human infection, *H. heilmanii* and *H. felis* have also been found to infect humans, but at lower frequencies than *H. pylori*.

Helicobacter infects over 50% of adult populations in developed countries, and nearly 100% in developing countries and some Pacific rim countries, making it one of the most prevalent infections of humans worldwide. Infection with *H. pylori* results in chronic stomach inflammation in all infected subjects, although the clinical gastroduodenal diseases associated with *Helicobacter* generally appear from several years to several decades after the initial infection. *H. pylori* is the causative agent of most peptic ulcers and chronic superficial (type B) gastritis in humans. *H. pylori* infection is also associated with atrophy of the gastric mucosa, gastric adenocarcinoma, and non-Hodgkin's lymphoma of the stomach (see, e.g., Blaser, J. Infect. Dis. 161:626-633, 1990; Scolnick *et al.*, Infect. Agents Dis. 1:294-309, 1993; Goodwin *et al.*, "*Helicobacter pylori*," *Biology and Clinical Practice*, CRC Press, Boca Raton, FL, 465 pp, 1993; Northfield *et al.*, "*Helicobacter pylori*," *Infection*, Kluwer Acad. Pub., Dordrecht, 178 pp, 1994).

If untreated, *H. pylori* infection and the associated gastritis persist lifelong, despite systemic and local immune responses to the bacterium in the infected host (Crabtree *et al.*, "Host responses," *in Helicobacter pylori Infection*, Northfield *et al.* (Eds.), Kluwer Acad. Pub., Dordrecht, pp. 40-52, 1991; Kist "Immunology of *Helicobacter pylori*," *in Helicobacter pylori in peptic ulceration and gastritis*, Marshall *et al.* (Eds.), Blackwell Sci. Pub., Oxford, pp. 92-110, 1991; Fox *et al.*, Infect. Immun. 61:2309-2315, 1993). Conventional treatment of peptic ulcer disease associated with *H. pylori* infection involves the use of one or more antibiotics combined with a proton pump inhibitor or an H_2-receptor antagonist. Such treatment regimens are unsuccessful in 10% to 70% of patients. Moreover, successful eradication of *H. pylori* infection with antibiotics does not prevent subsequent reinfection. The most effective conventional treatment is a triple therapy with bismuth, metronidazole, and either amoxicillin or tetracycline. The triple therapy treatment is complicated by a complex and prolonged dosing regimen, a high incidence of side-effects, poor compliance, and emergence of resistant bacterial strains (Hentschel *et al.*, N. Engl. J. Med. 328:308-312, 1993).

Summary of the Invention

We have identified a *Helicobacter* polypeptide (hereinafter designated "IgG 50 ligand"), which is recognized by monoclonal antibody IgG 50, and may be used, *e.g.*, in methods and compositions for preventing and/or treating *Helicobacter* infection. In addition, we have shown that monoclonal antibody IgG 50 is effective in imparting passive immunity against *Helicobacter* infection.

Accordingly, the invention features a method of preventing or treating *Helicobacter* (*e.g.*, *H. pylori*, *H. felis*, or *H. Heilmanii*) infection in a mammal involving administering (*e.g.*, to a mucosal (*e.g.*, oral or intranasal) surface) to the mammal a *Helicobacter* (*e.g.*, *H. pylori* or *H. felis*) antigen which is recognized by monoclonal antibody IgG 50 (*e.g.*, a *Helicobacter* antigen which is a polypeptide having a molecular weight of 16-19 kD, as measured by SDS-PAGE, *e.g.*, IgG 50 ligand, or a fragment or derivative thereof). Mammals that may be treated using the methods of the invention include, but are not limited to, mammals such as humans, cows, horses, pigs, dogs, cats, sheep, and goats.

The invention also features a substantially pure *Helicobacter* (*e.g.*, *H. pylori* or *H. felis*) polypeptide which is recognized by monoclonal antibody IgG 50, *e.g.*, a *Helicobacter* polypeptide having a molecular weight of 16-19 kD, as measured by SDS-PAGE, *e.g.*, IgG 50 ligand, or a fragment or derivative thereof. Vaccine compositions containing a polypeptide recognized by IgG 50 (or immunogenic fragments or derivatives thereof), as is described above, in a pharmaceutically acceptable carrier or diluent, are also included in the invention. The vaccine composition may also include an adjuvant (*e.g.*, a cholera toxin, the heat-labile enterotoxin of *Escherichia coli*, or a fragment or derivative thereof having adjuvant activity).

The invention also features a monoclonal antibody (*e.g.*, IgG 50) that recognizes a *Helicobacter* antigen (*e.g.*, a *Helicobacter* antigen which has a molecular weight of approximately 16-19 kD, as measured by SDS-PAGE, *e.g.*, IgG 50 ligand) which is recognized by monoclonal antibody IgG 50. Such a monoclonal antibody may be used in a method for preventing or treating *Helicobacter* infection in a mammal involving

administering (*e.g.*, to a mucosal surface, *e.g.*, orally) the monoclonal antibody to the mammal. Such an antibody may also be used in a method of detecting a *Helicobacter* antigen in a sample. In this method, the sample is contacted with the monoclonal antibody and detection of the antibody bound to the sample is used as an indication of the presence of the antigen in the sample. This method may employ standard immunological assays, *e.g.*, Western blot analysis or ELISA.

The invention also features a substantially pure nucleic acid (DNA or RNA) comprising a nucleotide sequence encoding a *Helicobacter* antigen recognized by monoclonal antibody IgG 50 (*e.g.*, a *Helicobacter* antigen which has a molecular weight of approximately 16-19 kD, as measured by SDS-PAGE, *e.g.*, IgG 50 ligand).

By "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation).

By "substantially pure" is meant a preparation which is at least 60% by weight (dry weight) the compound of interest, *e.g.*, a IgG 50 ligand polypeptide or IgG 50 ligand-specific antibody. Preferably the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, *e.g.*, column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "purified DNA" is meant DNA that is not immediately contiguous with both of the sequences (*e.g.*, coding sequences) with which it is immediately contiguous (one on the 5′ end and one on the 3′ end) in the naturally-occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into

an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first described.

Drawings

Fig. 1 is a graph showing the percentages of mice protected against *H. felis* challenge by passive oral immunization with the indicated monoclonal antibodies, including IgG 50 (50), which were raised against *H. felis* lysates. Protection was measured by urease broth assays carried out on gastric biopsies obtained one week after inoculation.

Fig. 2 is a photograph of a silver-stained SDS-polyacrylamide gel (12%) upon which the following samples were fractionated: lane A = molecular weight markers; lane B = *H. felis* lysate; lane C = an immunoprecipitate of an *H. felis* lysate using monoclonal antibody IgG 50; and lane D = a control immunoprecipitation reaction lacking antigen.

Methods and Compositions for Preventing and/or Treating *Helicobacter* Infection

The *Helicobacter* antigen recognized by monoclonal antibody IgG 50 (IgG 50 ligand) can be used in vaccination methods for preventing and/or treating *Helicobacter* (*e.g.*, *H. pylori*, *H. felis*, or *H. heilmanii*)

infection. In these methods, IgG 50 ligand, or an immunogenic fragment or derivative thereof, is administered to a mucosal (*e.g.*, intranasal, oral, ocular, gastric, rectal, vaginal, intestinal, or urinary tract) surface of a mammal, or is administered parenterally (*e.g.*, by intravenous, subcutaneous, intraperitoneal, or intramuscular routes). Any of a number of adjuvants that are known to one skilled in the art may be co-administered with the IgG 50 ligand vaccine antigen. For example, a cholera toxin (CT), the heat-labile enterotoxin of *Escherichia coli* (LT), or a fragment or derivative thereof having adjuvant activity, may be used in mucosal administration. An adjuvant such as RIBI (ImmunoChem, Hamilton, MT) or aluminum hydroxide may be used in parenteral administration. Recombinant attenuated vectors derived from microorganisms, *e.g.*, bacteria or viruses, such as Salmonella, Shigella, vaccinia virus, rotavirus, adenovirus, BCG virus, and poliovirus, may also be used for administration of the vaccines of the invention (see, *e.g.*, Lagranderie *et al.*, Vaccine 11:1283, 1993; Morris *et al.*, Gastroenterology 103:699-701, 1992; Hacket, Vaccine 8:5-11, 1990; Cardenas *et al.*, Clinical Microbiology Reviews 5:328-342, 1992; Dedieu *et al.*, J. Virol. 66:3161-3167, 1992; Jenkins *et al.*, J. Virol. 64:1201-1206, 1990; Karnell *et al.*, Vaccine 11:830-836, 1993; Schödel *et al.*, Sem. Immunol. 2:341-349, 1990; Tacket *et al.*, Vaccine 10:673-676, 1992).

IgG 50 ligand polypeptides which may be used in the vaccination methods of the invention may be prepared using any of several standard methods. For example, standard recombinant DNA methods may be employed (see, *e.g.*, Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., 1994). In these methods, a suitable host cell is transformed with

an appropriate expression vector containing all or part of an IgG 50 ligand-encoding nucleic acid (*e.g.*, DNA or RNA) fragment. Nucleic acids encoding IgG 50 ligand are isolated using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*). For example, monoclonal antibody IgG 50, which specifically recognizes IgG 50 ligand, may be used to immunoprecipitate IgG 50 ligand from *Helicobacter* lysates (see Fig. 2 and below). The immunoprecipitated polypeptide may be further purified by, *e.g.*, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), after which it is transferred onto a membrane (*e.g.*, a PVDF or nitrocellulose membrane), from which it is eluted for microsequence analysis (see, *e.g.*, Ausubel *et al.*, *supra*). Based on the amino acid sequence of the purified polypeptide, degenerate primers may be designed for use in polymerase chain reaction (PCR) methods for generating probes which may be used for screening *Helicobacter* (*e.g.*, *H. pylori* or *H. felis*) DNA libraries (*e.g.*, cDNA libraries), in order to isolate clones which encode IgG 50 ligand (see, *e.g.*, Ausubel *et al.*, *supra*).

In addition to immunoprecipitation and SDS-PAGE, other standard protein purification methods (*e.g.*, conventional column chromatography, high-performance liquid chromatography, and two-dimensional gel electrophoresis), and combinations of any of these methods, may be used to obtain purified IgG 50 ligand for microsequence analysis. A nucleic acid clone encoding IgG 50 ligand may also be obtained by screening an expression library, *e.g.*, a λGT11 expression library, prepared from *Helicobacter* (*e.g.*, *H. pylori* or *H. felis*) nucleic acid (see, *e.g.*, Blanchard *et al.*, Infection and Immunity 63(4):1394-1399, 1995; Ausubel *et al.*, *supra*).

Any of a variety of expression systems may be used to produce recombinant IgG 50 ligand polypeptides, once

nucleic acid molecules which encode them are obtained. For example, IgG 50 ligand polypeptides may be produced in a prokaryotic host (*e.g.*, *E. coli*) or in a eukaryotic host (*e.g.*, yeast cells (*e.g.*, *Saccharomyces cerevisiae*), mammalian cells (*e.g.*, COS1, NIH3T3, or JEG3 cells), or arthropod cells (*e.g.*, *Spodoptera frugiperda* (SF9) cells)). Such cells are available from a number of different sources known to those skilled in the art, *e.g.*, the American Type Culture Collection (ATCC), Rockville, MD (also see, *e.g.*, Ausubel *et al.*, *supra*). The transfection/transformation method used, and the choice of expression vector, will depend on the host system selected, as is described by, *e.g.*, Ausubel *et al.*, *supra*. Expression vectors (*e.g.*, plasmid or viral vectors) can be chosen from, *e.g.*, those described in *Cloning Vectors: A Laboratory Manual* (Pouwels *et al.*, 1985, Supp. 1987; also see, *e.g.*, Ausubel *et al.*, *supra*).

IgG 50 ligand polypeptides, particularly short fragments, may also be produced by chemical synthesis, *e.g.*, by the method described in *Solid Phase Peptide Synthesis*, 1984, 2nd ed., Stewart and Young, eds., Pierce Chemical Co., Rockford, IL, and by standard *in vitro* translation methods. In addition, IgG 50 ligand may be purified from *Helicobacter* cultures, using standard methods.

In addition to native, full length, *Helicobacter* IgG 50 ligand, polypeptide fragments of IgG 50 ligand, or IgG 50 ligand polypeptides (or polypeptide fragments of IgG 50 ligand) containing mutations, may be used in the invention, provided that antigenicity is retained. Fragments of IgG 50 ligand polypeptides are made by standard methods, including, *e.g.*, recombinant, chemical synthetic, or proteolytic methods (see, *e.g.*, Ausubel *et al.*, *supra*). Generally, IgG 50 ligand fragments should be at least 10 amino acids in length, for example 50-200

amino acids in length, in order to maintain antigenicity. Genes encoding fragments of IgG 50 ligand, and/or IgG 50 ligand containing mutations, are made using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*). Fragments and derivatives of IgG 50 ligand which are included in the invention may be screened for antigenicity using standard methods in the art, *e.g.*, by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity (see below).

Fusion proteins containing IgG 50 ligand (or a fragment or derivative thereof) fused to, *e.g.*, an adjuvant (*e.g.*, CT, LT, or a fragment or derivative thereof having adjuvant activity), are also included in the invention, and can be prepared using standard methods (see, *e.g.*, Ausubel *et al.*, *supra*). In addition, the vaccines of the invention may be covalently coupled or cross-linked to adjuvants (see, *e.g.*, Cryz *et al.*, Vaccine 13:67-71, 1994; Liang *et al.*, J. Immunology 141:1495-1501, 1988; and Czerkinsky *et al.*, Infection and Immunity 57:1072-1077, 1989).

The amount of vaccine administered depends on, *e.g.*, the particular vaccine antigen, whether an adjuvant is co-administered with the antigen, the type of adjuvant co-administered, the mode and frequency of administration, and the desired effect (*e.g.*, protection and/or treatment), as can be determined by one skilled in the art. In general, the vaccine antigens of the invention are administered in amounts ranging between, *e.g.*, 1 μg and 100 mg. If adjuvants are administered with the vaccines, amounts ranging between, *e.g.*, 1 ng and 1 mg may be used. Administration is repeated as necessary, as can be determined by one skilled in the art. For example, a priming dose can be followed by 3 booster doses at weekly intervals.

Antibodies against IgG 50 ligand (*e.g.*, monoclonal antibodies such as IgG 50) may be used in passive immunization methods for protecting and/or treating mammals (*e.g.*, humans) from *Helicobacter* (*e.g.*, *H. pylori*, *H. felis*, or *H. heilmanii*) infection. Monoclonal antibodies against IgG 50 ligand are produced using standard immunological methods (see, *e.g.*, Coligan *et al.*, Eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, New York, 1994). Antigens for use in these methods may be obtained, *e.g.*, by immunoprecipitation of *Helicobacter* lysates with IgG 50, followed by fractionation of the immunoprecipitate by SDS-PAGE. Antibodies of any isotype, *e.g.*, IgA and IgG, may be used in the invention. In addition to monoclonal antibodies, purified polyclonal antibodies, single chain antibodies, chimeric antibodies (*e.g.*, human/murine chimeric antibodies), humanized antibodies (*e.g.*, humanized murine monoclonal antibodies), and Fab fragments which recognize IgG 50 ligand may be used in the invention.

In the passive immunization methods of the invention, antibodies (*e.g.*, monoclonal antibodies) which recognize IgG 50 ligand, *e.g.*, IgG 50, are administered to a mucosal (*e.g.*, oral or intragastric) surface of a mammal. The amount of antibody used in this method can be determined by one skilled in the art.

The IgG 50 ligand polypeptides, nucleic acids, and antibodies of the invention may also be used for detecting the presence of anti-*Helicobacter* antibodies, *Helicobacter* nucleic acids, or *Helicobacter* polypeptides, respectively, in biological samples, using standard methods (*e.g.*, Western blot analysis, ELISA, and nucleic acid hybridization methods; see, *e.g.*, Ausubel *et al.*, *supra*; Coligan *et al.*, *supra*).

The following examples are meant to illustrate, but not to limit, the methods of the invention. Modifications of the conditions and parameters set forth below that are apparent to one skilled in the art are included in the invention.

EXAMPLES

Example I - Production and Isolation of Monoclonal Antibodies Against *H. Felis*

The *H. felis* mouse model is an accepted model for *H. pylori* infection of humans (see, *e.g.*, Lee *et al.*, European Journal of Gastroenterology and Hepatology 7:303-309, 1995). This model has been used to identify other candidate *Helicobacter* vaccine antigens, *e.g.*, urease (Czinn *et al.*, Vaccine 11:637-642, 1993; Blanchard *et al.*, *supra*). In order to identify and characterize *Helicobacter* antigens that may be useful vaccines, monoclonal antibodies against sonicated *H. felis* lysates were generated, as follows.

Monoclonal antibodies (including IgG 50) against sonicated *H. felis* lysates were produced by a modification of the procedure of Mazanec *et al.* (J. Virol. 61:2624-2626, 1987; Czinn *et al.*, *supra*). BALB/c mice obtained from the Jackson Laboratory (Bar Harbor, Maine) were immunized intragastrically four times over a 6-week period. The first three times, the mice were immunized with 2 mg of sonicated *H. felis* plus 10 μg cholera toxin (Sigma Chemical Co., St. Louis, MO). For the last immunization, the cholera toxin was omitted, and, in addition to the intragastric immunization, the mice received an intravenous boost of 2 mg of *H. felis* sonicate. Three days following the final immunization, the mice were sacrificed, and their spleen cells were hybridized to SP2/01-Ag myeloma cells (ATCC accession number CRL 8006), using standard methods (see, *e.g.*,

Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Coligan *et al.*, *supra*). Clones obtained by limited dilution were screened for secretion of anti-*H. felis* monoclonal antibodies by an enzyme-linked immunosorbent assay (ELISA) using an *H. felis* outer-membrane preparations (OMPs) as an antigen (Blanchard *et al.*, *supra*). Several monoclonal antibodies which recognize *H. felis* antigens, including IgG 50, were isolated using this method.

Example II - Passive Protection Against *H. felis* Challenge Imparted by Monoclonal Antibodies Against *H. felis*

Monoclonal antibodies which recognize *Helicobacter* antigens, and were isolated using the methods described above, were analyzed in passive protection studies, as described by Czinn *et al.* (*supra*) and Blanchard *et al.* (*supra*). Briefly, *H. felis* harvested from a fresh plate was brought to a concentration of 5 x 10^6 colony-forming units (CFU)/ml in phosphate-buffered saline (PBS). Two ml aliquots of the bacteria were combined with 2 ml of ascites fluid containing the anti-*H. felis* monoclonal antibodies, and incubated at 37°C for 30 minutes. Four hundred µl (~10^6 organisms) of the bacteria/antibody mixture was administered to mice by gastric intubation. The mice received an additional 200 µl of ascites fluid at 4, 8, and 24 hours. Mice were necropsied on day 9, and their gastric tissues were examined for *H. felis* colonization by urea broth assays (see, *e.g.*, Blanchard *et al.*, *supra*). Using these methods, monoclonal antibody IgG 50 was found to be effective at passively protecting mice from *H. felis* challenge (see Fig. 1 and Table 1, below).

Monoclonal antibody IgG 50 is produced by hybridoma cell line #50-G_6-B_7, which was deposited with

the ATCC (Rockville, MD) on June 30, 1995, and assigned ATCC accession number HB-11952.

Table 1. Passive protection assay data. (Sv255 = an anti-Sendai virus IgA monoclonal antibody; titer = amount of antibody administered)

monoclonal antibody		# infected 12h	# infected 24h	titer	% infected
Expt. 1:	IgG 50	0/7	1/7	$10^{4.5}$	14
	IgG 52	4/7	6/7	$10^{5.0}$	86
	IgG 84	5/7	5/7	$10^{4.5}$	71
	IgG 40	0/7	1/7	$10^{5.0}$	14
	Sv255	5/5	5/5	-----	100
Expt. 2:	IgG 40	0/7	0/7	$10^{5.0}$	0
	Sv255	5/5	5/5	---	100
	IgA 34	7/7	7/7	$10^{4.5}$	100
	IgA 109	7/7	7/7	$10^{4.5}$	100
	IgA 251	7/7	7/7	$10^{4.0}$	100
	IgA 394	7/7	7/7	---	100

Example III - Characterization of the *Helicobacter* Antigen Recognized by IgG 50

In order to characterize further the *Helicobacter* antigen (IgG 50 ligand) recognized by IgG 50, radiolabeled *H. felis* OMPs were immunoprecipitated with IgG 50, and the immunoprecipitates were analyzed by SDS-PAGE (12%), followed by autoradiography (Blanchard *et al.*, *supra*). These experiments revealed that IgG 50 recognizes a *Helicobacter* polypeptide of approximately 16-19 kD molecular weight (Blanchard *et al.*, *supra*; Fig. 2, lane C). Further characterization of IgG 50 ligand by metal chelate chromatography has revealed that it binds nickel.

IgG 50 immunoprecipitates two bands (Fig. 2, lane C) from *H. felis* lysates which are close to each other in molecular weight. Amino acid analysis of the polypeptides in these bands shows that their amino acid contents are very similar (Table 2 (upper band) and Table 3 (lower band)).

Mol Percent Report

Sample ID: UPPERBAND BASELINE CORRECTED

Sampling Interval: 1.0 sec

Data Start : 0.00 min
Data Duration : 20.00 min
Peak Ht Threshold : 1000 uAU

Int. Std. Amt : 250 pmol

Calibration File : PEIRCE
Reference Time : 0.00 min
Reference Offset 1: 0.00 min
Reference Offset 2: 0.00 min

(No ISTD Peak Specified)

Integration Interval: 0.0 to 20.0 min

PEAK ID	RET. TIME min	PMOL BY HEIGHT	PMOL correc. INT STD	MOL %
Aspartic Acid	6.30	43.37	0.00	3.15
Glutamic Acid	6.73	181.72	0.00	13.19
Serine	7.35	161.61	0.00	11.73
Glycine	7.73	218.27	0.00	15.84
Histidine	8.08	20.68	0.00	1.50
Arginine	8.68	55.10	0.00	4.00
Threonine	8.97	44.09	0.00	3.20
Alanine	9.35	73.14	0.00	5.31
Proline	9.70	31.80	0.00	2.31
Tyrosine	12.53	34.92	0.00	2.53
Valine	13.72	41.76	0.00	3.03
Methionine	14.03	21.19	0.00	1.54
Cysteine	15.58	44.02	0.00	3.20
Isoleucine	16.23	34.26	0.00	2.49
Leucine	16.47	75.20	0.00	5.46
NORLEUCINE	16.82	193.62	0.00	14.05
Phenylalanine	17.20	38.86	0.00	2.82
Lysine	18.28	64.05	0.00	4.65

TOTAL PMOLS RECOVERED 1377.67

Minimum Peak Threshold: 1000 uAU (52 peaks below threshold)
(32 peaks found)
(18 peaks matched)

Table 2

Mol Percent Report

Sample ID: LOWERBAND BASELINE CORRECTED

Data Start : 0.00 min
Data Duration : 20.00 min
Peak Ht Threshold : 1000 uAU

Sampling Interval: 1.0 sec

Int. Std. Amt : 250 pmol

Calibration File : PEIRCE
Reference Time : 0.00 min
Reference Offset 1: 0.00 min
Reference Offset 2: 0.00 min

(No ISTD Peak Specified)

Integration Interval: 0.0 to 20.0 min

PEAK ID	RET. TIME min	PMOL BY HEIGHT	PMOL correc. INT STD	MOL %
Aspartic Acid	5.30	88.75	0.00	3.42
Glutamic Acid	6.73	330.41	0.00	12.73
Serine	7.35	318.93	0.00	12.28
Glycine	7.73	470.17	0.00	18.11
Histidine	8.00	40.59	0.00	1.56
Arginine	8.67	118.61	0.00	4.57
Threonine	8.97	94.00	0.00	3.62
Alanine	9.35	138.23	0.00	5.32
Proline	9.63	43.34	0.00	1.67
Tyrosine	12.52	84.79	0.00	3.27
Valine	13.70	92.46	0.00	3.56
Methionine	14.05	53.17	0.00	2.05
Cysteine	15.57	74.55	0.00	2.87
Isoleucine	16.23	79.83	0.00	3.08
Leucine	16.47	157.95	0.00	6.08
NORLEUCINE	16.80	214.99	0.00	8.28
Phenylalanine	17.18	81.17	0.00	3.13
Lysine	18.27	114.24	0.00	4.40

TOTAL PMOLS RECOVERED 2596.19

Minimum Peak Threshold: 1000 uAU (53 peaks below threshold)
(33 peaks found)
(18 peaks matched)

Table 3

Other embodiments are in the following claims.

What is claimed is:

1. A substantially pure *Helicobacter* polypeptide which is recognized by monoclonal antibody IgG 50.

2. The polypeptide of claim 1, wherein said polypeptide has a molecular weight of approximately 16-19 kD, as measured by SDS-PAGE.

3. A vaccine comprising the polypeptide of claim 1, or an immunogenic fragment or derivative thereof, in a pharmaceutically acceptable carrier or diluent.

4. The vaccine of claim 3, further comprising an adjuvant.

5. The vaccine of claim 4, wherein said adjuvant is a cholera toxin, or a fragment or derivative thereof having adjuvant activity.

6. The vaccine of claim 4, wherein said adjuvant is the heat-labile enterotoxin of *Escherichia coli*, or a fragment or derivative thereof having adjuvant activity.

7. A composition for treating *Helicobacter* infection in a mammal, said composition comprising a monoclonal antibody that recognizes a *Helicobacter* antigen recognized by IgG 50, formulated for administration.

8. The composition of claim 7, wherein said mucosal surface is oral.

9. The composition of claim 7, wherein said monoclonal antibody is IgG 50.

10. A monoclonal antibody that recognizes a *Helicobacter* antigen recognized by monoclonal antibody IgG 50.

11. The monoclonal antibody of claim 10, wherein said *Helicobacter* antigen has a molecular weight of approximately 16-19 kD, as measured by SDS-PAGE.

12. The monoclonal antibody of claim 10, wherein said monoclonal antibody is IgG 50.

13. A substantially pure nucleic acid comprising a nucleotide sequence encoding a *Helicobacter* antigen recognized by monoclonal antibody IgG 50.

14. A method of detecting a *Helicobacter* antigen in a sample, said method comprising the steps of:

(i). contacting said sample with the monoclonal antibody of claim 10; and

(ii). detecting said antibody bound to said sample as an indication of the presence of said antigen in said sample.

Fig. 1

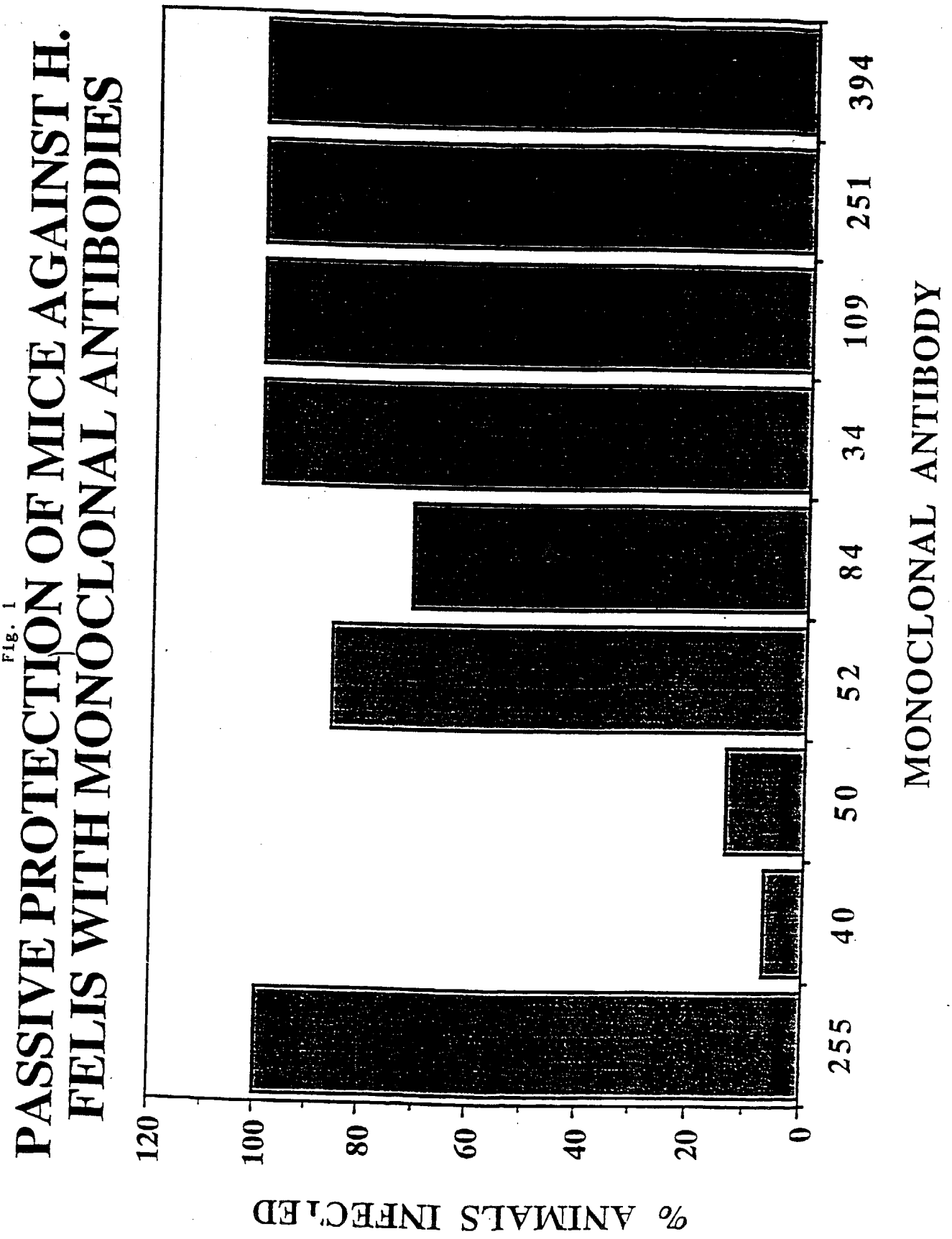
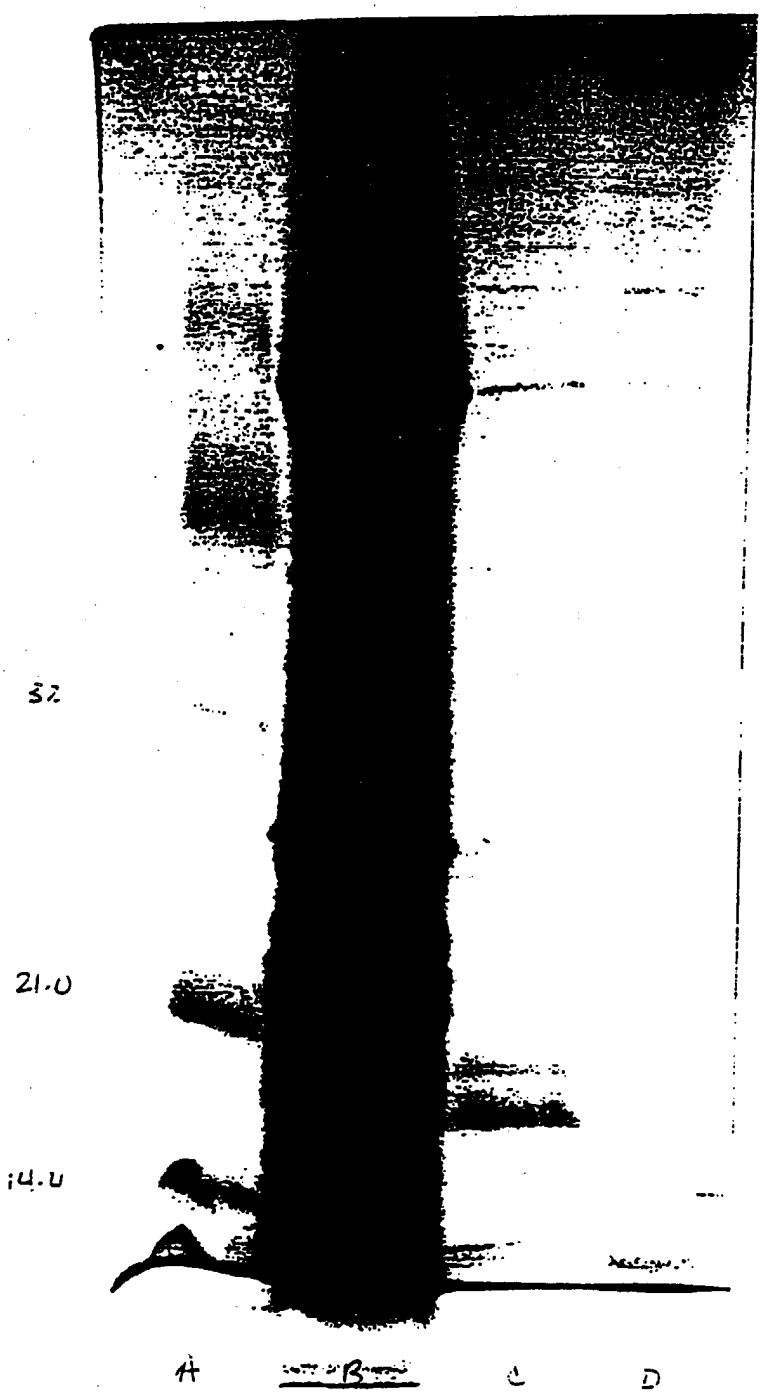

PASSIVE PROTECTION OF MICE AGAINST H. FELIS WITH MONOCLONAL ANTIBODIES
% ANIMALS INFECTED
0
20
40
60
80
100
120
255
40
50
52
84
34
109
251
394
MONOCLONAL ANTIBODY

Fig. 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11245

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CZINN et al. Protection of germ-free mice from infection by Helicobacter felis after active oral or passive IgA immunization. Vaccine. April 1993, Vol.11(6), pages 637-642, see pages 637-640, see whole article.	3-5, 7-12 6
Y	CLAYTON et al. Molecular cloning and expression of Campylobacter pylori species specific antigens in Escherichia coli K-12. Infection and Immunity. February 1989, Vol.57(2), pages 623-629, especially page 625, see whole article.	13
X	US 5,262,156 A (ALEMOHAMMAD) 16 November 1993, see entire document, especially column 2, lines 21-68.	1-2
A	RAPPUOLI et al. Development of a vaccine against Helicobacter pylori: a short review. European journal of Gastroenterology and Hepatology. 1993, Vol. 5(suppl. 2), pages 576-578, see whole article.	1-12
Y	PAVLOVSKIS et al, Adjuvant effect of escherichia coli heat-labile enterotoxin on host immune response following vaccination with non-viable campylobacter antigens. International workshop on Campylobacter, Helicobacter and related organisms. 7-10 October 1991, S10, see whole abstract.	3-4, 6-8
Y	CLEMENTS et al. Adjuvant activity of Escherichia coli heat-labile enterotoxin and effect on the induction of oral tolerance in mice to unrelated protein antigens. Vaccine. June 1988, Vol. 6, pages 269-277, see whole article.	3-6
A	McGHEE et al. The mucosal immune system from fundamenal concepts to vaccine development. Vaccine. 1992, Vol. 10(2), pages 75-88, see whole article.	3-9